High-throughput downstream processing using cellulose fiber chromatography and ÄKTA™ chromatography systems

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Introduction

Developing a process or looking for a clone of interest often requires screening large numbers of samples. Many analyses require purified proteins, and often substantial hands-on time is required for purification. The novel cellulose fiber technology (known as Fibro) may be used with ÄKTA chromatography systems and autosamplers to reduce process time significantly compared with conventional chromatography. Fibro chromatography enables residence times of seconds, with full chromatographic runs in a few minutes per cycle. Here, we describe a versatile solution that significantly increases throughput by completing the purification process in minutes rather than hours.

What is Fibro chromatography?

GE Healthcare Life Sciences Fibro technology (also known as Puridify technology) utilizes the high flow rates and high capacities of cellulose fiber. Fibro technology offers improved protein capture compared to conventional chromatography, with residence times measured in seconds rather than minutes (Fig 1 and Fig 2).

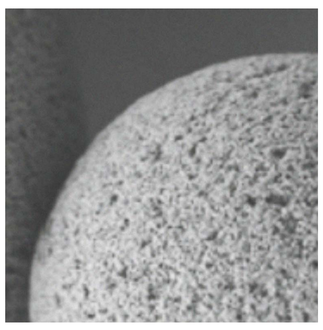

Fig 1. Conventional chromatography: diffusive flow. Mass transfer is restricted by the slow diffusion of molecules through the pores in the bead, and process flow is restricted by the rigidity of the beads.

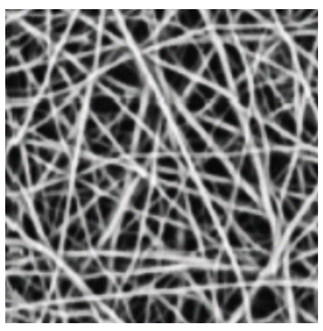

Fig 2. Fibro chromatography: convective flow. Enables fast mass transfer by convective flow while process flow is only restricted by the size of the pores in the matrix material.

A full chromatographic mAb capture cycle can be completed in a few minutes

A 0.4 mL laboratory-scale Fibro unit can run a full chromatographic cycle in a few minutes (Fig 3). With a simple plug-and-play setup, bidirectional flow, and high multicycle lifetime, the laboratory-scale Fibro unit is a suitable scouting tool for understanding a process and for optimizing conditions—or for generating hundreds of milligrams of material when cycled. Dynamic binding capacity (DBC) of the protein A Fibro unit at different residence times is shown in Figure 4.

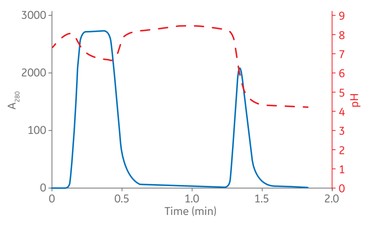

Fig 3. Protein A bind-elute profile on clarified cell culture mAb feed of 3.8 mg/mL purified on a 0.4 mL protein A laboratory-scale Fibro unit: 1.6 s residence time, 1.85 min total run time, 96% recovery, 211 ppm host cell protein (HCP).

Laboratory-scale Fibro unit

- Development tool
- 0.4 mL adsorbent volume
- Connect to syringe or ÄKTA chromatography system

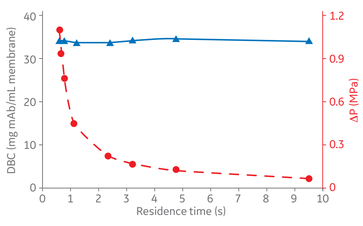

Fig 4. Flow-rate independent binding properties of a 0.4 mL protein A Fibro unit. DBC was determined by loading 1.5 mg/mL purified mAb to 10% breakthrough.

Rapid multicycling while maintaining high recovery

Remaining dynamic binding capacity (DBC) for 250 cycles without cleaning in place (CIP) included in each cycle is shown in Figure 5. If desired, a CIP procedure can be performed between each sample to avoid cross-contamination and to ensure no pressure increase.

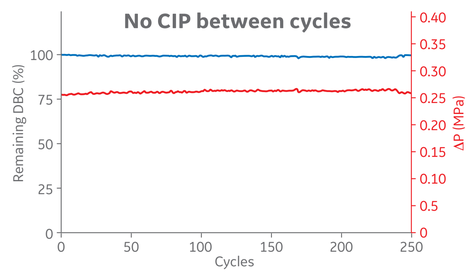

Fig 5. DBC performance of protein A Fibro unit over 250 cycles. Sample: clarified cell culture fluid with a mAb titer of 3.8 mg/mL, with no cleaning in place (CIP) carried out between runs. A flow rate of 15 mL/min was used for all phases on a 0.2 mL adsorbent volume, 2 min/cycle, to a 30 mg/mL load challenge.

Significantly reduced process development time compared to conventional chromatography

Process development time can be significantly reduced due to the short cycling times delivered by Fibro chromatography. Compared to conventional chromatography coumns, PreDictor™ RoboColumn™ units decrease process development time and material consumption to a large extent. Fibro chromatography further reduces process development time. In contrast to PreDictor RoboColumn units, full chromatograms are provided, giving improved quality monitoring.

A comparison of the optimization of the mAb capture step is shown in Table 1.

Table 1. Optimization study of the chromatography purification process comparing the HiTrap™ MabSelect SuRe™ 1 mL run on ÄKTA avant 25, PreDictor RoboColumn run on Tecan Freedom Evo™ 200, and laboratory-scale Fibro units run on ÄKTA avant 25 chromatography system

	HiTrap column	PreDictor RoboColumn	Cellulose Fibro unit
Samples per run	1	8	1
Time per run (min)	~ 180	~ 180	~ 5
Runs needed for purifying 12 samples	12	2	12
Total time (min)	~ 2160	~ 360	~ 60
Protein recovered (mg)	~ 20 to 25	~ 10 to 15	~ 8 to 10
Recovery	> 90%	> 90%	> 90%

Data source: “Linking AMBR to HTPD chromatography for assessment of cell line clone manufacturability” presented at ACS conference at New Orleans, 2018.

Manage large numbers of samples with automation

It is simple and quick to run many samples in serial mode when running the Fibro units on an ÄKTA system connected to an autosampler. A method can be set up in the UNICORN™ software and automatically repeated, matching the number of samples loaded in the autosampler.

- 84* samples may be run without intervention using Fibro units connected to an ÄKTA chromatography system with autosampler.
- Run time for each Fibro unit is 4 min†. Conventional run time is 60 min‡.
- Fibro units may be used with existing ÄKTA systems without modification such as adding new valves, filters etc.
- Real-time UV, conductivity, and optional pH detection for every sample.

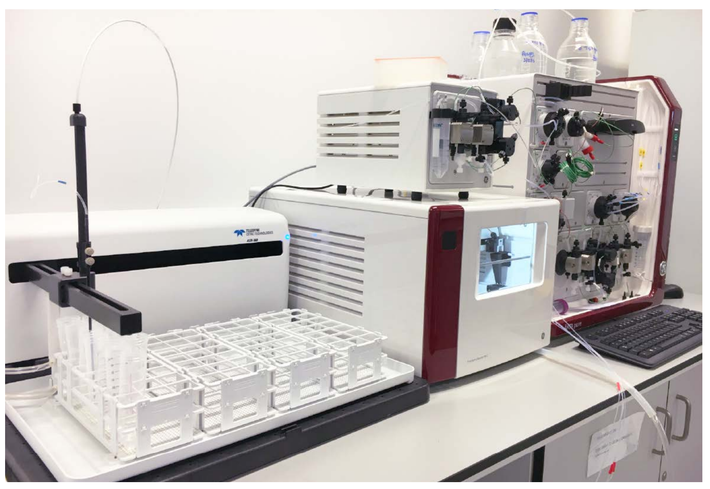

Fig 6. This picture illustrates a set-up facilitating high-throughput purification using the autosampler ASX-560 (left), sample pump (middle top), fraction collector (middle bottom), and ÄKTA pure 25 system (right).

Conclusions

We describe an attractive tool for early-stage development that will ultimately reduce time-to-product and cost:

- Fibro technology delivers a full chromatographic mAb capture cycle in minutes. The first solution that will be available will be a laboratory-scale Fibro unit with immobilized MabSelect™ PrismA ligand.
- Large sample volumes can be loaded at high flow rate while maintaining high DBC.
- Serial setup with autosampler on ÄKTA systems provides full chromatograms in high-throughput mode, managing large numbers of samples in an automated way.

* Up to 8 racks each with 21 tubes = 168 samples with no CIP. With CIP between samples, the number decreases to 84.
† Assuming 50 mL sample with a run speed of 16 mL/min.
‡ Compared to 1 mL chromatography column.

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